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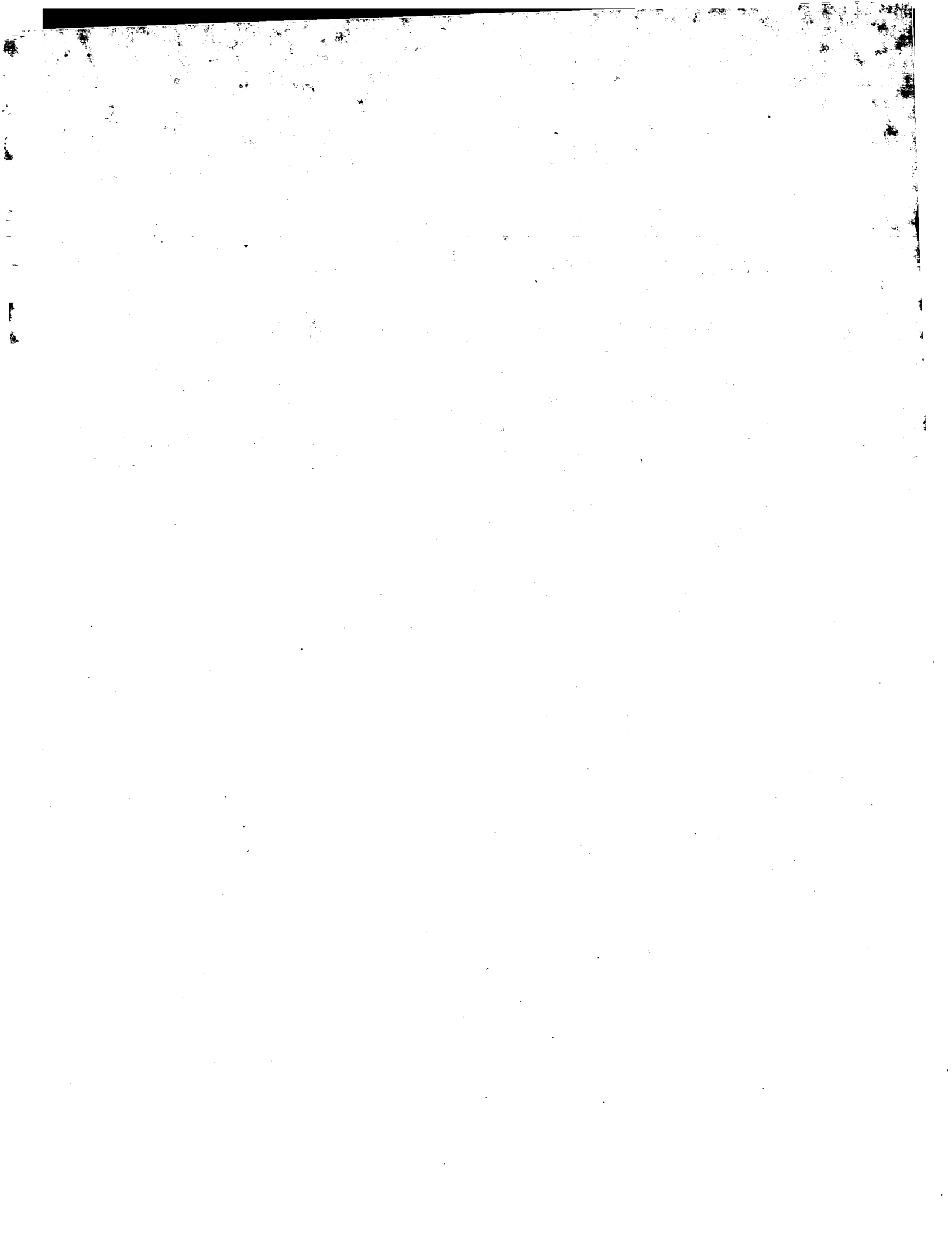
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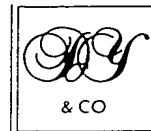


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Dated

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P01/7700 0.00-0101447.1

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**0101447.1**

**19 JAN 2001**

3. Full name, address and postcode of the  
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The University of Edinburgh  
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South Bridge  
Edinburgh  
EH8 9YL

Patents ADP number (if you know it)

If the applicant is a corporate body, give  
the country/state of its incorporation

GS 64165001

4. Title of the invention Regulation of Glucocorticoid Concentration

5. Name of your agent (if you have one) D YOUNG & CO

"Address for service" in the United Kingdom  
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*D Young & Co*

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**D YOUNG & CO**

19 Jan 2001

Agents for the Applicants

12. Name and daytime telephone number of person to contact in the United Kingdom

Antonio Maschio

023 80634816

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# Regulation of Glucocorticoid Concentration

The present invention relates to the regulation of glucocorticoid levels. In particular, the invention relates to the regulation of intracellular glucocorticoid levels in macrophages to enhance the successful resolution of the inflammatory response mediated by such cells.

Glucocorticoids such as cortisol have a number of diverse effects on different body tissues. Our International Patent Application WO 90/04399 was concerned with the problem that therapeutically administered cortisol tends to be converted in the body to inactive cortisone by 11 $\beta$ -hydroxysteroid dehydrogenase enzymes (11 $\beta$ -HSDs). Our earlier invention provided for the potentiation of cortisol by the administration of an inhibitor of the 11 $\beta$ -dehydrogenase activity of these enzymes.

It is also known that the reverse reaction, converting inactive cortisone to active cortisol, is accomplished in certain organs by 11 $\beta$ -reductase activity of these enzymes. This activity is also known as corticosteroid 11 $\beta$ -reductase, cortisone 11 $\beta$ -reductase, or corticosteroid 11 $\beta$ -oxidoreductase.

Expression of 11 $\beta$ -HSD1 in a range of cell lines encodes either a bi-directional enzyme [Agarwal AK, Monder C, Eckstein B & White PC J Biol Chem 264, 18939-18943 (1989); Agarwal AK, Tusie-Luna M-T, Monder C & White PC Mol Endocrinol 4, 1827-1832 (1990)] or a predominant 11 $\beta$ -reductase [Duperrex H, Kenouch S, Gaeggeler HP, et al. Endocrinology 132, 612-619 (1993); Jamieson PM, Chapman KE, Edwards CRW & Seckl JR. Endocrinology 136, 4754-4761 (1995)] which, far from inactivating glucocorticoids, regenerates active 11 $\beta$ -hydroxysteroid from otherwise inert 11-keto steroid. 11 $\beta$ -reductase activity, best observed in intact cells, activates 11-keto steroid to alter target gene transcription and differentiated cell function [Duperrex H, Kenouch S, Gaeggeler HP, et al. Endocrinology 132, 612-619 (1993); Low SC, Chapman KE, Edwards CRW & Seckl JR Journal of Molecular Endocrinology 13, 167-174 (1994)]. 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 are the products of different genes and share only 20% amino acid homology [Agarwal AK, Mune T, Monder C & White PC (1994) J Biol Chem 269, 25959-25962 (1994); Albiston AL, Obeyesekere VR, Smith RE & Krozowski ZS Mol Cell Endocrinol 105, R11-R17 (1994)]. In our International patent application WO97/07789, the contents of which and documents referenced therein being incorporated herein by

reference, we discuss the inhibition of 11 $\beta$ -reductase activity in vivo, and the treatment of many of the deleterious effects of glucocorticoid excess.

Cortisol promotes hepatic gluconeogenesis by several mechanisms, including antagonism of the effects of insulin on glucose transport, and interactions with insulin and glucose in the regulation of several enzymes which control glycolysis and gluconeogenesis. These include glucokinase, 6-phosphofructokinase, pyruvate kinase, phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase. Inhibiting production of cortisol from cortisone in the liver therefore enhances hepatic glucose uptake and inhibits hepatic glucose production by several mechanisms. Moreover, the influence of inhibiting 11 $\beta$ -reductase activity in the liver of patients with insulin resistance or glucose intolerance may be greater than in healthy subjects because in insulin resistance or deficiency the influence of cortisol on PEPCK has been shown to be greater; obese patients secrete more cortisol; insulin resistant patients are more sensitive to glucocorticoids; and insulin down-regulates 11 $\beta$ -HSD1 expression so that 11 $\beta$ -reductase activity may be enhanced in conditions of insulin resistance or deficiency.

Our International patent application WO97/07789 also shows that 11 $\beta$ -HSD1 is expressed in rat adipose tissue and in adipocyte cell lines in culture, where it converts 11-dehydrocorticosterone to corticosterone (the rat equivalents of human cortisone and cortisol, respectively). This suggests that similar 11 $\beta$ -reductase activity will be observed in human adipose tissue, with the result that inhibition of the enzyme will result in alleviation of the effects of insulin resistance in adipose tissue in humans. This would lead to greater tissue utilisation of glucose and fatty acids, thus reducing circulating levels. The invention therefore provides, in a further aspect, the use of an inhibitor of 11 $\beta$ -reductase in the manufacture of a medicament for increasing insulin sensitivity in adipose tissue.

It is also known that glucocorticoid excess potentiates the action of certain neurotoxins, which leads to neuronal dysfunction and loss. We have studied the interconversion between 11-dehydrocorticosterone and corticosterone in rat hippocampal cultures, and have found (surprisingly in view of the damaging effects of glucocorticoids) that 11 $\beta$ -reductase activity dominates over 11 $\beta$ -dehydrogenase activity in intact hippocampal cells. The reason for this activity is unknown, but this result indicates that glucocorticoid excess may be controlled in hippocampal cells (and by extension in the nervous system in general) by use of an 11 $\beta$ -reductase inhibitor, and the invention therefore provides in

an alternative aspect the use of an inhibitor of  $11\beta$ -reductase in the manufacture of a medicament for the prevention or reduction of neuronal dysfunction and loss due to glucocorticoid potentiated neurotoxicity. It is also possible that glucocorticoids are involved in the cognitive impairment of ageing with or without neuronal loss and also in dendritic attenuation. Furthermore, glucocorticoids have been implicated in the neuronal dysfunction of major depression. Thus an inhibitor of  $11\beta$ -reductase could also be of value in these conditions.

Our earlier International patent application, therefore, provides that the beneficial effects of inhibitors of  $11\beta$ -reductase are many and diverse, and it is envisaged that in many cases a combined activity will be demonstrated, tending to relieve the effects of endogenous glucocorticoids in diabetes mellitus, obesity (including centripetal obesity), neuronal loss and the cognitive impairment of old age. However, the effects of glucocorticoids on macrophages are not described.

### Summary of the Invention

We have now determined that glucocorticoid activity in macrophages stimulates the termination of the inflammatory response to reach a successful outcome. Our studies indicate that glucocorticoid (GC) treatment specifically enhances the non-inflammatory phagocytosis of apoptotic neutrophils (PMN) by macrophages. Moreover, the potentiation of  $11\beta$ -HSD1 activity in macrophages increases intracellular glucocorticoid levels to achieve the same beneficial effects.

According to a first aspect of the present invention, therefore, we provide the use of a modulator of glucocorticoid metabolism in the manufacture of a composition for the potentiation of a successful resolution of an inflammatory response in a mammal.

The modulator in accordance with the invention preferably increases the intracellular concentration of active glucocorticoid in macrophages. In an advantageous embodiment, this can be achieved by increasing the intracellular activity of  $11\beta$ -HSD1 reductase, either by administering  $11\beta$ -HSD1 itself or by administering a modulator of  $11\beta$ -HSD1 reductase activity.

In a second embodiment, the invention provides an engineered macrophage in which endogenous active glucocorticoid levels have been increased. This may be achieved,

for example, by genetically engineering the macrophages, such as to increase 11 $\beta$ -HSD1 activity therein. The macrophages according to the invention are useful in the treatment of conditions in which inflammatory responses are advantageously managed to a successful resolution.

5

Glucocorticoids themselves may also be used in accordance with the present invention. Particularly inactive forms of glucocorticoid, such as dehydrocorticosterone, which are converted to active forms by 11 $\beta$ -HSD1 or equivalent enzymes, are useful as substrates which can be administered to sites of inflammation and converted *in situ* by  
10 macrophages to active glucocorticoid. Glucocorticoid may be administered in combination with a modulator of glucocorticoid metabolism, or with an engineered macrophage in accordance with the above aspects of the invention.

15

Thus, the invention provides at least two of a glucocorticoid and/or an engineered macrophage and/or a modulator of glucocorticoid metabolism, as described above, for separate, simultaneous separate or sequential use in the potentiation of a successful resolution of the inflammatory response in a mammal.

20

Moreover, the invention provides a pharmaceutical composition comprising one or more of a glucocorticoid and/or an engineered macrophage and/or a modulator of glucocorticoid metabolism, as described above.

25

In a further aspect, there is provided a method of potentiating a successful inflammatory response in a mammal, comprising administering to a mammal in need thereof a composition comprising a glucocorticoid and/or an engineered macrophage and/or a modulator of glucocorticoid metabolism, as described above.

## Detailed Description of the Invention

30

### Definitions

35

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, molecular genetics, nucleic acid chemistry, hybridisation techniques and biochemistry). Standard techniques are used for molecular, genetic and biochemical methods (see generally, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed. (1989) Cold

Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and Ausubel et al., Short Protocols in Molecular Biology (1999) 4th Ed, John Wiley & Sons, Inc. which are incorporated herein by reference) and chemical methods.

5 A "modulator of glucocorticoid metabolism" is any compound, substance or treatment which upregulates or downregulates the activity (concentration) of glucocorticoid in a cell. Advantageously, the cell is a macrophage. The activity of the glucocorticoid is preferably increased in the macrophage, for example by increasing the biosynthesis of active glucocorticoid or the conversion of inactive forms of glucocorticoid to active  
10 glucocorticoid. Thus, for example, the modulator may increase the levels of  $11\beta$ -HSD1 in the macrophage, which is shown in the present invention to lead to advantageous effects in the phagocytosis of apoptotic cells and thus the successful resolution of an inflammatory response. The modulator may be exogenously administered  $11\beta$ -HSD1 itself, or a nucleic acid encoding  $11\beta$ -HSD1 which is delivered to the cell such that it may  
15 be expressed therein to produce increased levels of  $11\beta$ -HSD1.

A "successful resolution of the inflammatory response" is an inflammatory response in which the desired outcome to prevent the occurrence of chronicity (phagocytosis of apoptotic cells) is increased or otherwise potentiated. It is not synonymous with anti-  
20 inflammatory treatment. The invention potentiates, that is better achieves the benefits of, the natural inflammatory response; it does not avoid an inflammatory response, but assists its purpose and aids its prompt resolution.

An "engineered" macrophage is a macrophage which has been modified in order to  
25 increase the levels of active glucocorticoid therein. This may be achieved, in a preferred embodiment, by engineering the macrophage to express increased levels of  $11\beta$ -HSD1. This enzyme acts as a reductase in the macrophage and increases the conversion of inactive glucocorticoid to its active form. Methods for engineering macrophages to produce elevated levels of  $11\beta$ -HSD1 are known to those skilled in the art and further  
30 described below.

#### Glucocorticoids

Glucocorticoids are a group of adrenocortical steroid hormones whose metabolic effects  
35 include stimulation of gluconeogenesis, increased catabolism of proteins, and mobilisation of free fatty acids; they are also known to be potent inhibitors of the

inflammatory response (allergic response). The vast majority of glucocorticoid activity in most mammals is from cortisol, also known as hydrocortisone. Corticosterone is the major glucocorticoid in rodents. Synthetic glucocorticoids are also known, such as dexamethasone. Cortisol binds to the glucocorticoid receptor in the cytoplasm and the hormone-receptor complex is then translocated into the nucleus, where it binds to its DNA response elements and modulates transcription of relevant genes.

Glucocorticoid receptors are universally present and as a consequence, these steroid hormones have a huge number of effects on physiological systems. The best known and studied effects of glucocorticoids are on carbohydrate metabolism and immune function. Indeed, the name glucocorticoid derives from early observations that these hormones were involved in glucose metabolism. In the fasting state, cortisol stimulates several processes that collectively serve to increase and maintain normal concentrations of glucose in blood.

Glucocorticoids are known to have potent anti-inflammatory and immunosuppressive properties. This is particularly evident when they administered at pharmacological doses, but also is important in normal immune responses. As a consequence, glucocorticoids are widely used as drugs to treat chronic (unnecessarily persistent) inflammatory conditions such as arthritis, nephritis, asthma or dermatitis, and as adjunct therapy for conditions such as autoimmune diseases.

Some of the steroid drugs for topical administration for anti-inflammatory purposes include Betamethasone (Diprolene® cream), Clobetasol (Temovate®), Desonide (Desowen®), Fluocinolone (Derma-Smoother/FS®), Fluocinonide (Lidex®), Hydrocortisone (Anusol®, Cortaid®, Hydrocortone®), Mometasone (Elocon®) and Triamcinolone (Aristocort®, Knaflage®).

Glucocorticoids circulate in inactive forms, which are reduced to active compounds at the site of action. 9- $\alpha$ -Fluorinated 11-dehydrosteroids like 11-dehydro-dexamethasone (DH-D) are rapidly activated by human kidney 11 $\beta$ -HSD type II to the active dexamethasone (D). Moreover, hepatic 11 $\beta$ -HSD1 is known to reduce cortisone to cortisol in the liver. Thus, in the context of the present invention, an active glucocorticoid is the reduced form, such as cortisol or dexamethasone; and inactive glucocorticoid is, for example, cortisone or 11-dehydro-dexamethasone.

### Modulators of Glucocorticoid Metabolism

In a preferred embodiment, such modulators are enzymes which catalyse the conversion of inactive glucocorticoids to active glucocorticoids. Thus, the invention is particularly concerned with 11 $\beta$ -HSD reductase enzymes. For example, the human 11 $\beta$ -HSD2 enzyme is known and details thereof may be found at GenBank Accession No. M76661.1 GI:179469. The sequence of 11 $\beta$ -HSD1 may be found at Accession no. NM\_005525.1 GI:5031764. Modulators of the activity of such enzymes are also to be considered modulators of glucocorticoid metabolism; thus, compounds such as carbenoxolone, which inhibits 11 $\beta$ -HSD1, are encompassed by the invention, as are potentiators of 11 $\beta$ -HSD1 activity. See Monder and White, (1993) Vitamins and Hormones 47:187.

### Engineered Macrophages

The engineering of macrophages to modify the activity of a modulator of glucocorticoid metabolism may be carried out by conventional genetic engineering techniques. Typically this will involve transfer of a nucleic acid vector encoding the modulator in a recombinant replicable vector. The vector may be used to replicate the nucleic acid in a compatible host cell. Suitable host cells include bacteria such as E. coli, yeast, mammalian cell lines and other eukaryotic cell lines, for example insect Sf9 cells.

A polynucleotide encoding a modulator according to the invention in a vector is operably linked to a control sequence that is capable of providing for the expression of the coding sequence by the host cell, i.e. the vector is an expression vector. The term "operably linked" means that the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under condition compatible with the control sequences.

The control sequences may be modified, for example by the addition of further transcriptional regulatory elements to make the level of transcription directed by the control sequences more responsive to transcriptional modulators.

Vectors of the invention may be transformed or transfected into macrophages to provide for expression of the modulator therein.

The vectors may be for example, plasmid or virus vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable  
 5 marker genes, for example a neomycin resistance gene.

Control sequences operably linked to sequences encoding the protein of the invention include promoters/enhancers and other expression regulation signals. These control sequences may be selected to be compatible with the host cell for which the expression  
 10 vector is designed to be used in. The term "promoter" is well-known in the art and encompasses nucleic acid regions ranging in size and complexity from minimal promoters to promoters including upstream elements and enhancers.

The promoter is typically selected from promoters which are functional in mammalian  
 15 cells, although prokaryotic promoters and promoters functional in other eukaryotic cells may be used. The promoter is typically derived from promoter sequences of viral or eukaryotic genes. For example, it may be a promoter derived from the macrophage, such as the CD68 promoter [Adenovirus-mediated gene transfer of a secreted form of human macrophage scavenger receptor inhibits modified low-density lipoprotein  
 20 degradation and foam-cell formation in macrophages. Laukkanen J, Lehtolainen P, Gough PJ, Greaves DR, Gordon S, Yla-Herttuala S. CIRCULATION 101: (10) 1091-1096 (2000)] (so that 11 $\beta$ HSD1 expression is directed in circulating monocytes and their progeny) or the Mouse macrophage metalloelastase (MME) promoter [Induction and regulation of macrophage metalloelastase by hyaluronan fragments in mouse  
 25 macrophages. Horton MR, Shapiro S, Bao C, Lowenstein CJ, Noble PW. JOURNAL OF IMMUNOLOGY 162: (7) 4171-4176 APR 1 1999] (so that 11 $\beta$ HSD1 expression is activated as monocytes move into inflamed sites). The promoter may be a promoter that functions in a ubiquitous manner (such as promoters of  $\alpha$ -actin,  $\beta$ -actin, tubulin) or, alternatively, a tissue-specific manner. Tissue-specific promoters specific for  
 30 macrophages are particularly preferred. They may also be promoters that respond to specific stimuli, for example promoters that bind steroid hormone receptors. Viral promoters may also be used, for example the Moloney murine leukaemia virus long terminal repeat (MMLV LTR) promoter, the Rous sarcoma virus (RSV) LTR promoter or the human cytomegalovirus (CMV) IE promoter.

It may also be advantageous for the promoters to be inducible so that the levels of expression of the heterologous gene can be regulated during the life-time of the cell. Inducible means that the levels of expression obtained using the promoter can be regulated.

5

In addition, any of these promoters may be modified by the addition of further regulatory sequences, for example enhancer sequences. Chimeric promoters may also be used comprising sequence elements from two or more different promoters described above.

10 Techniques for transformation of macrophages are known in the art, and include DNA transfection techniques such as electroporation or lipofection and viral transduction. Uptake of naked nucleic acid constructs by mammalian cells is enhanced by several known transfection techniques for example those including the use of transfection agents. Example of these agents include cationic agents (for example calcium  
15 phosphate and DEAE-dextran) and lipofectants (for example lipofectam<sup>TM</sup> and transfectam<sup>TM</sup>). Typically, nucleic acid constructs are mixed with the transfection agent to produce a composition.

## 20 Formulation of Pharmaceutical Compositions

A pharmaceutical composition according to the invention is a composition of matter comprising an inactive glucocorticoid as active ingredient. The active ingredients of a pharmaceutical composition comprising the combination according to the invention are  
25 contemplated to exhibit excellent therapeutic activity in the successful resolution of inflammatory responses. Dosage regima may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

30

The active compound may be administered in a convenient manner such as by the topical, oral, intravenous (where water soluble), intramuscular, subcutaneous, intranasal, intradermal or suppository routes or implanting (e.g. using slow release molecules). Depending on the route of administration, the active ingredient may be required to be  
35 coated in a material to protect said ingredients from the action of enzymes, acids and other natural conditions which may inactivate said ingredient.

A topical formulation may be liposome-based. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes.

- 5 The active compound may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.
- 10 The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the
- 15 contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required
- 20 particle size in the case of dispersion and by the use of surfactants.

The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic

25 agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the

30 required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilisation. Generally, dispersions are prepared by incorporating the sterilised active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable

35 solutions, the preferred methods of preparation are vacuum drying and the freeze-drying

technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

Tablets, troches, pills, capsules and the like may also contain the following: a binder  
5 such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium  
phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the  
like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose,  
lactose or saccharin may be added or a flavouring agent such as peppermint, oil of  
wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may  
10 contain, in addition to materials of the above type, a liquid carrier.

Various other materials may be present as coatings or to otherwise modify the physical  
form of the dosage unit. For instance, tablets, pills, or capsules may be coated with  
shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a  
15 sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring  
such as cherry or orange flavour. Of course, any material used in preparing any dosage  
unit form should be pharmaceutically pure and substantially non-toxic in the amounts  
employed. In addition, the active compound may be incorporated into sustained-release  
preparations and formulations.

20 As used herein "pharmaceutically acceptable carrier and/or diluent" includes any and all  
solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and  
absorption delaying agents and the like. The use of such media and agents for  
pharmaceutical active substances is well known in the art. Except insofar as any  
25 conventional media or agent is incompatible with the active ingredient, use thereof in the  
therapeutic compositions is contemplated. Supplementary active ingredients can also be  
incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form  
30 for ease of administration and uniformity of dosage. Dosage unit form as used herein  
refers to physically discrete units suited as unitary dosages for the mammalian subjects  
to be treated; each unit containing a predetermined quantity of active material calculated  
to produce the desired therapeutic effect in association with the required pharmaceutical  
carrier. The specification for the novel dosage unit forms of the invention are dictated by  
35 and directly dependent on (a) the unique characteristics of the active material and the  
particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of

compounding such as active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired.

5 The principal active ingredients are compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

10 In a further aspect there is provided the combination of the invention as hereinbefore defined for use in the treatment of disease which involves an inflammatory response, such as peritonitis.

15 The invention is further described below, with reference to the following non-limiting examples.

### Examples

#### Example 1

20 Phagocytosis of apoptotic neutrophils by macrophages

Culture of neutrophils (PMN) under appropriate conditions leads to apoptosis and ingestion of apoptotic cells by macrophages, leading to non-necrotic clearing of neutrophil matter. Failure to clear such apoptotic neutrophil material in a timely manner leads to deleterious chronic inflammation and ongoing tissue damage. Using 200nM dexamethasone, we observed a marked increase in the uptake of apoptotic neutrophils by macrophages. This is shown in Figure 1. Glucocorticoid action increases both the numbers of macrophages involved in phagocytosis and the number of PMN ingested per macrophage.

30

#### Example 2

Expression of 11 $\beta$ -HSD1 in macrophages

Figure 2 shows an RT-PCR of transcripts obtained from kidney, liver and macrophages. 35 The expression of 11 $\beta$ -HSD1 in macrophages can clearly be observed, as can the absence of 11 $\beta$ -HSD2 expression.

### Example 3

11 $\beta$ -HSD1 converts inactive glucocorticoid to active glucocorticoid

- 5 In Figure 3, the results of an experiment are shown in which the conversion of inactive glucocorticoid (A) to active glucocorticoid (B) is monitored. 11 $\beta$ -HSD1 clearly shows conversion of (A) to (B) over a 24 hour period. However, as shown in Figure 4, this effect can be blocked by high concentrations of the 11 $\beta$ -HSD1 inhibitor carbenoxolone.
- 10 The effect is also observed in phagocytosis by peripheral macrophages, as shown in figure 5. Active glucocorticoid raises the phagocytosis performance of macrophages two-fold over 24 hours, as does inactive glucocorticoid after incubation (to allow activation by 11 $\beta$ -HSD1). However, incubation of macrophages with inactive glucocorticoid in the presence of the 11 $\beta$ -HSD1 inhibitor carbenoxolone completely
- 15 abolishes the effects thereof. The activation of inactive glucocorticoid correlates with the production of 11 $\beta$ -HSD1 in macrophages during macrophage differentiation, as shown in Figures 6 and 7, and bone marrow differentiation, as shown in Figure 8.

- All publications mentioned in the above specification are herein incorporated by
- 20 reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications
- 25 of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

**Claims**

1. Use of a modulator of glucocorticoid metabolism in the manufacture of a composition for the potentiation of a successful resolution of an inflammatory response in a mammal.
2. Use according to claim 1, wherein the modulator increases the intracellular concentration of glucocorticoids in macrophages.
3. Use according to claim 1 or claim 2, wherein the modulator is a modulator of the activity of an  $11\beta$ -HSD1 reductase enzyme.
4. An engineered macrophage having increased endogenous biosynthesis of active glucocorticoid.
5. A macrophage according to claim 4 which is genetically engineered.
6. A genetically engineered macrophage according to claim 5, wherein endogenous  $11\beta$ -HSD1 activity is upregulated.
7. A macrophage according to any one of claims 4 to 6, for use in the potentiation of a successful resolution of the inflammatory response in a mammal.
8. Use of a glucocorticoid in the manufacture of a composition for the potentiation of a successful resolution of the inflammatory response in a mammal.
9. Use according to claim 8, wherein the glucocorticoid is activated by  $11\beta$ -HSD1.
10. Use according to claim 8 or claim 9, wherein the glucocorticoid is administered in an inactive form.
11. Use according to claim 10, wherein the glucocorticoid is a dehydroxycorticosterone.

12. Use according to any one of claims 8 to 11, wherein the composition further comprises a modulator of glucocorticoid metabolism according to any one of claims 1 to 3.
- 5 13. A method of potentiating a successful resolution of the inflammatory response in a mammal, comprising administering to a mammal in need thereof a composition comprising a glucocorticoid.
- 10 14. A method according to claim 13, wherein the glucocorticoid is activated by  $11\beta$ -HSD1.
- 15 15. A method according to claim 13 or claim 14, wherein the glucocorticoid is administered in an inactive form.
- 15 16. A method according to claim 15, wherein the glucocorticoid is a dehydroxycorticosterone.
- 20 17. A method according to any one of claims 13 to 16, wherein the composition further comprises a modulator of glucocorticoid metabolism according to any one of claims 1 to 3.
18. A pharmaceutical composition comprising a glucocorticoid in inactive form.
- 25 19. A pharmaceutical composition according to claim 18, wherein the glucocorticoid is a dehydroxycorticosterone.
20. A pharmaceutical composition according to claim 18 or claim 19, wherein the glucocorticoid is activated by  $11\beta$ -HSD1.
- 30 21. A pharmaceutical composition according to any one of claims 18 to 20, wherein the composition further comprises a modulator of glucocorticoid metabolism according to any one of claims 1 to 3.

**Abstract**

The invention relates to use of a modulator of glucocorticoid metabolism, or the administration of inactive glucocorticoids, for the potentiation of a successful resolution of an inflammatory response in a mammal.

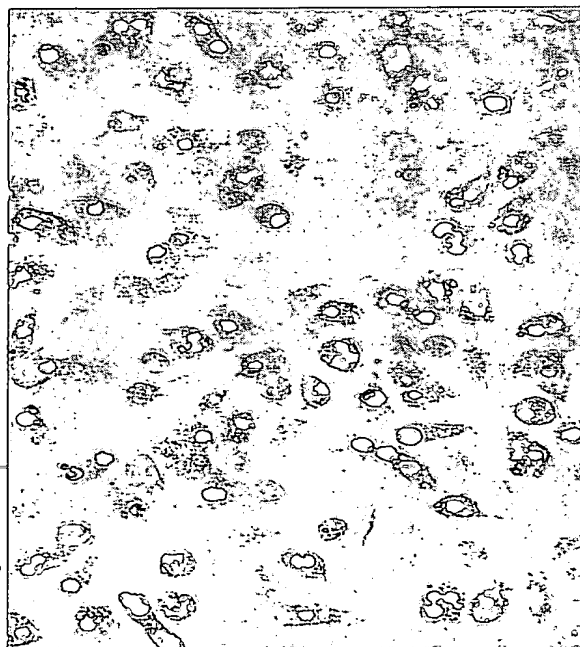
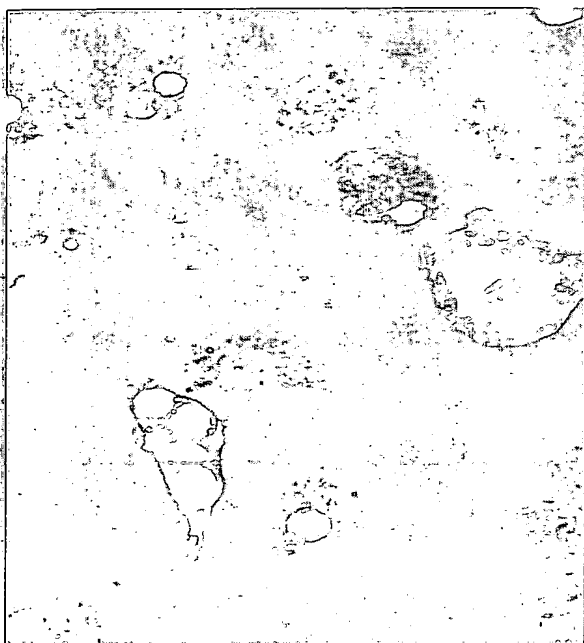
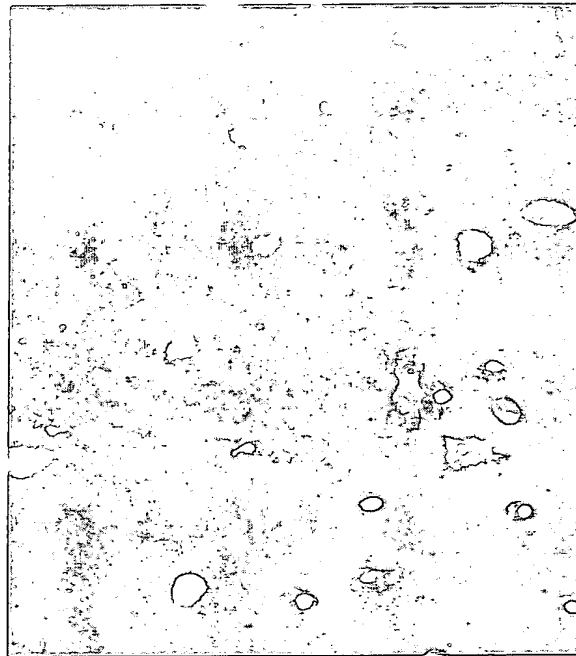
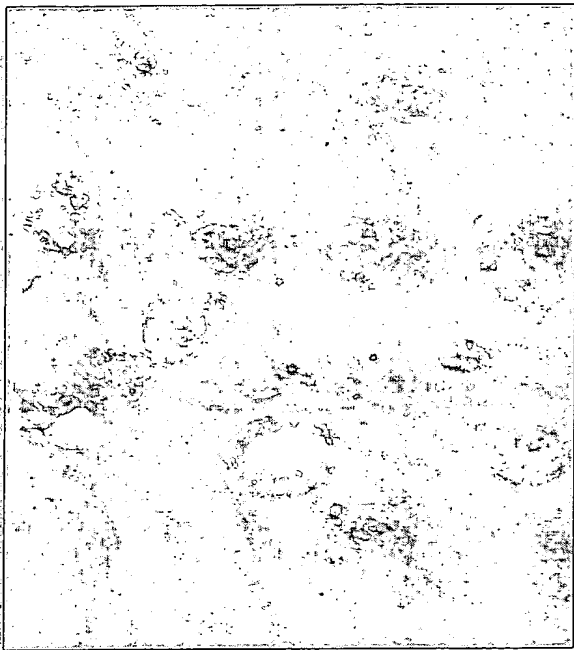
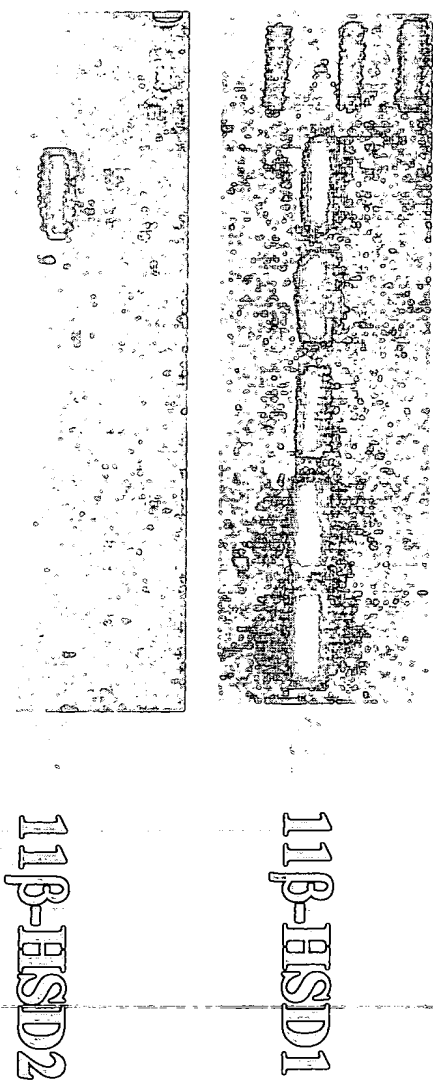




FIGURE 2

Macrophages are 11 $\beta$ -HSD1 specific





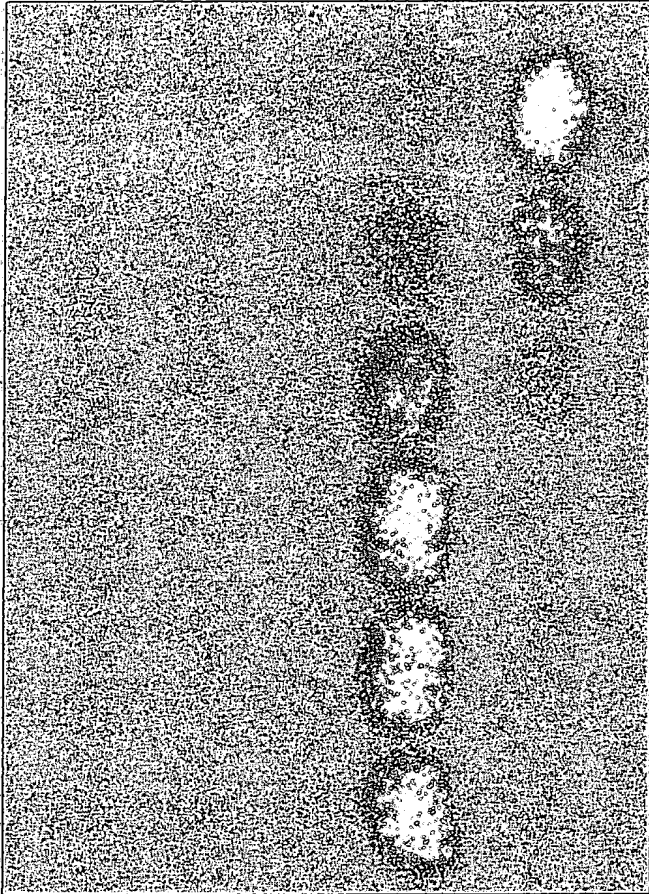
11 $\beta$ HSD-1 Bioassay



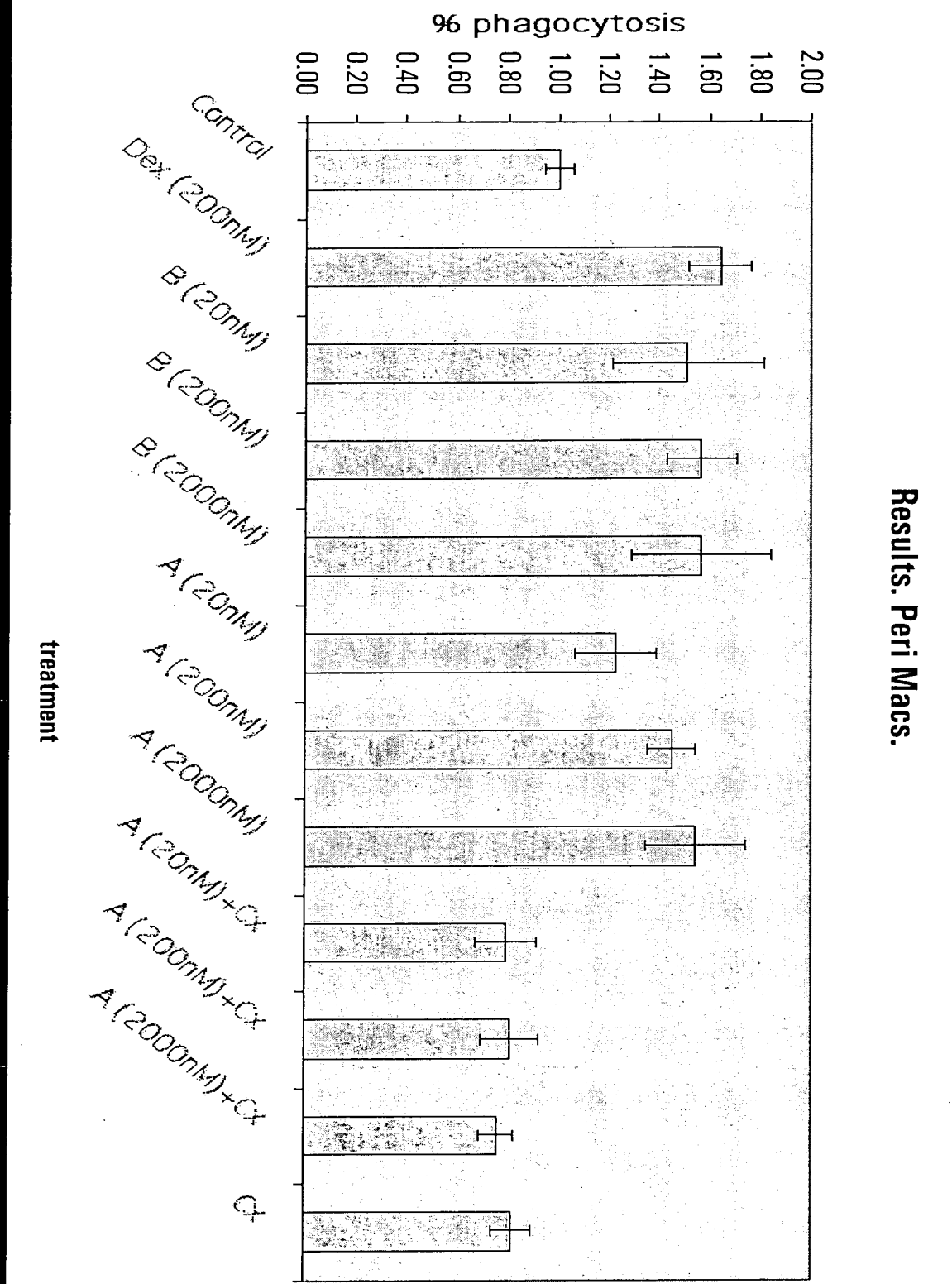
FIGURE 4

11 $\beta$ HSD Activity after 24hrs  
Carbenoxolone treatment





FIGURE 5



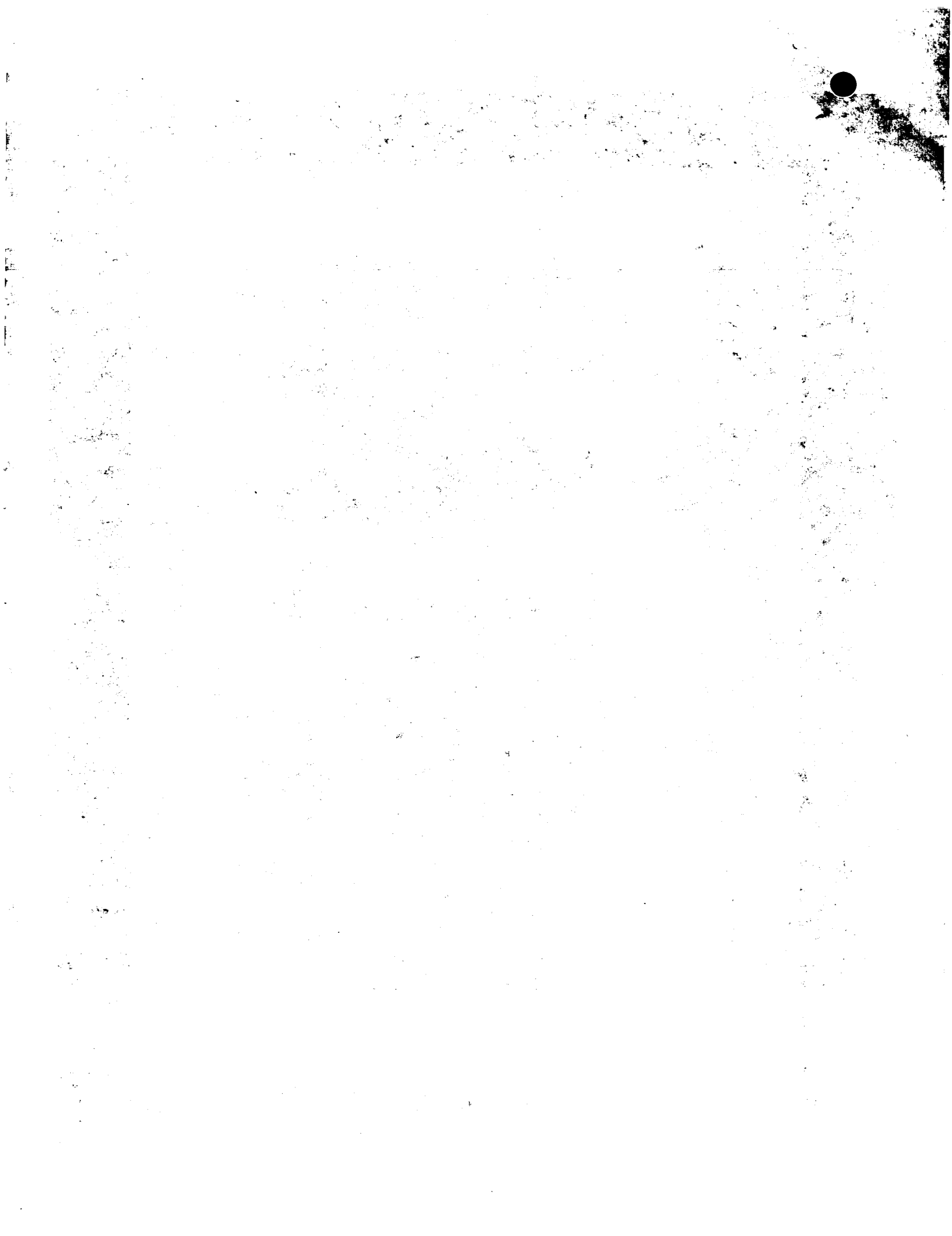


FIGURE 6

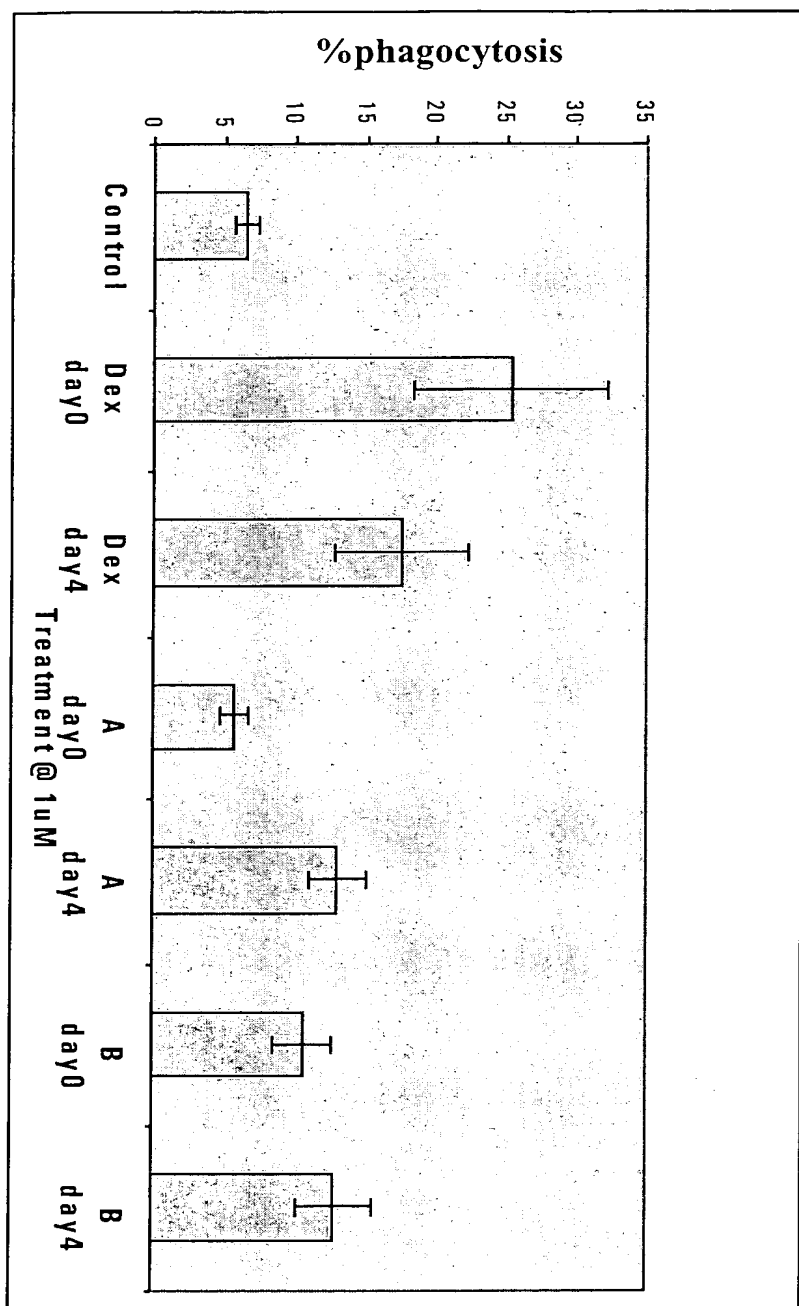
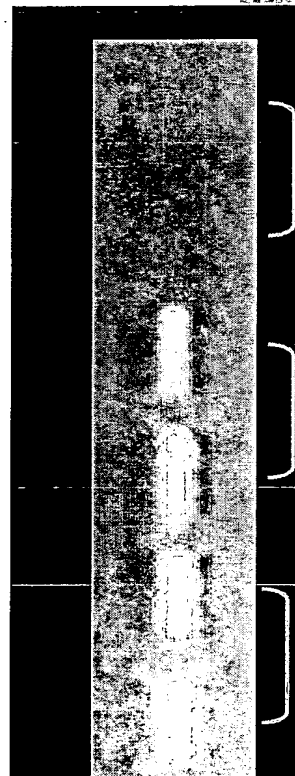
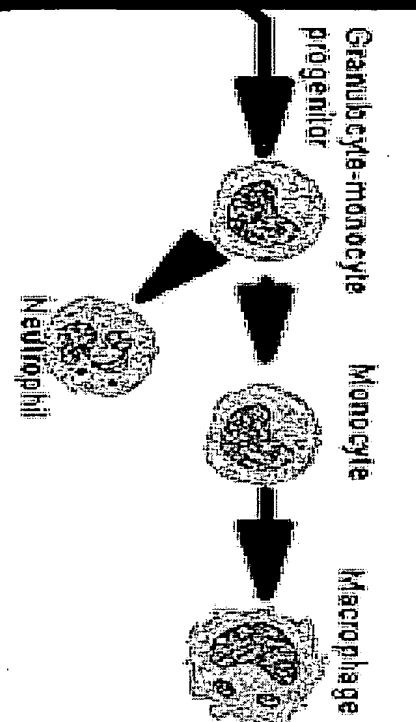




FIGURE 7

# $11\beta$ HSD-1 activity during murine bone marrow differentiation

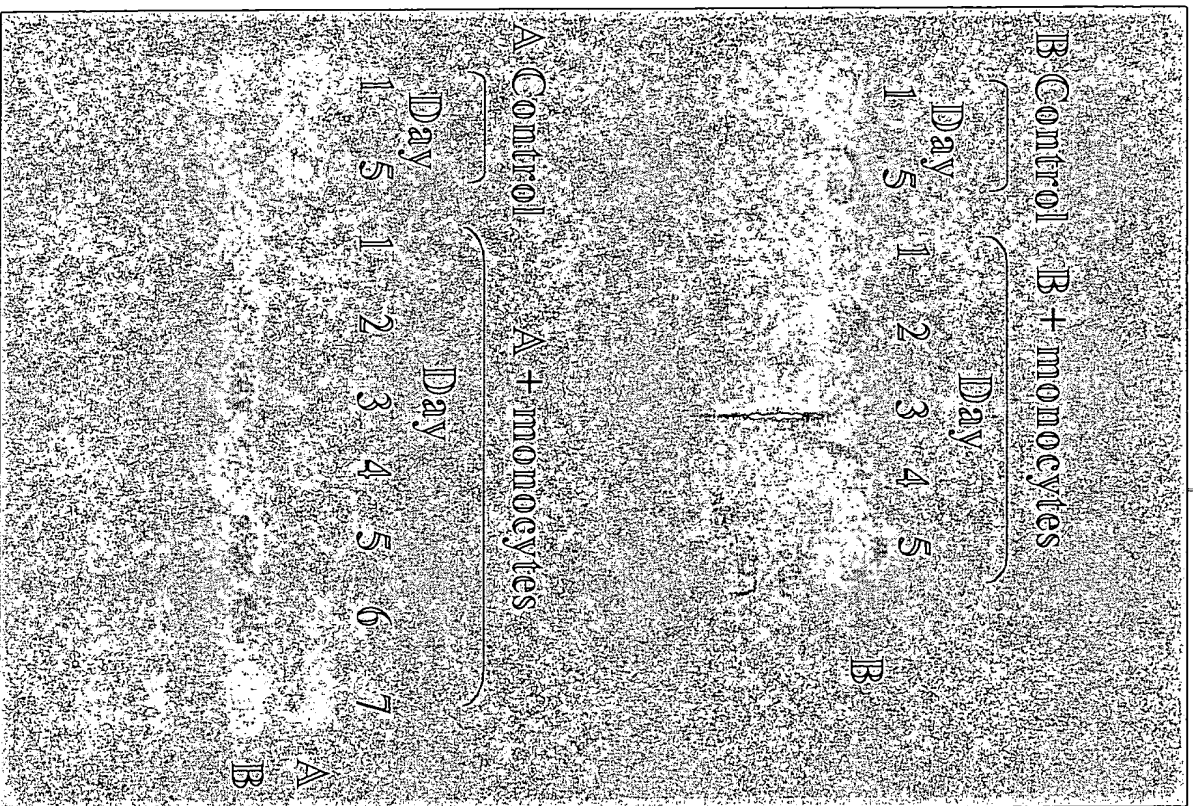




FIGURE 8

# 11 $\beta$ HSD-1 activity during differentiation

